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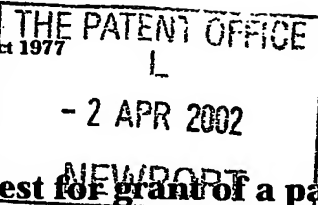
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2 APR 2002

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UNITED KINGDOM

Patents ADP number (if you know it)

7112386002 ✓

If the applicant is a corporate body, give the country/state of its incorporation

ENGLAND AND WALES

4. Title of the invention

PROTEIN

5. Name of your agent (if you have one)

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PROTEIN

The present invention relates to the use of Solute Carrier 6 (SC6), a taurine transporter, in the diagnosis, screening, treatment and prophylaxis of hypoxia related conditions e.g. cancer. Compositions comprising the protein, including vaccines and antibodies that are immunospecific for the protein and agents that modulate the protein expression or activity are also provided.

Solute Carrier 6

The solute carrier 6 gene, accession no. NM_003043, was cloned and characterised by Ramamoorthy S, *et al.*, 1994 and encodes the human taurine transporter protein. The cDNA was isolated from a human placental cDNA library and is highly related to the rat brain taurine transporter. Transfection of this cDNA into HeLa cells results in a marked elevation of taurine transport activity. The activity of the cDNA-induced transporter is dependent on the presence of Na⁺ as well as Cl⁻. The Na⁺/Cl⁻/taurine stoichiometry for the cloned transporter is 2:1:1. The transporter is specific for taurine and other beta-amino acids, including beta-alanine, and exhibits high affinity for taurine (Michaelis-Menten constant approximately 6 microM). The nucleotide sequence of the coding region predicts a 620-amino acid protein with a calculated M(r) of 69,853 Da, (Ramamoorthy S, *et al.*, (1994) Biochem J, 300 (Pt 3), 893-900).

Cellular function of Taurine

Taurine (2-aminoethane sulphonic acid), a ubiquitous beta-amino acid is conditionally essential in man. It is derived from methionine and cysteine and is not utilized in protein synthesis but found free or in some simple peptides. Intracellular taurine is generally maintained at high concentrations although its' role appears to be cell-specific. Plasma taurine levels are also high, although decreases have been observed in response to surgical injury and numerous pathological conditions including cancer and sepsis (Stapleton PP, *et al.*, (1998) JPEN J Parenter Enteral Nutr, Jan-Feb, 22(1), 42-8).

Taurine is an inhibitory amino acid and a known neuromodulator. One key function is as an osmolyte in most cells. Thus, it may regulate many biological processes, including heart rhythm, contractile function, blood pressure, platelet aggregation, neuronal excitability, body temperature, learning, motor behavior, food consumption, eye sight, sperm motility, cell proliferation and viability, energy metabolism and bile acid synthesis. Many of these actions are associated with alterations in either ion transport or protein phosphorylation. Although the effects on ion transport have been attributed to changes in membrane structure, they could be equally affected by a change in the activity of the affected transporters. The transporter activity can be altered by enhanced protein expression, changes in the phosphorylation status of the protein and cytoskeletal changes. Interestingly, all three events are altered by osmotic stress (Schaffer S, *et al.*, (2000) Amino Acids, 19(3-4), 527-46).

Additionally, taurine has been associated with hypoxia, hypoglycemia, ischemia, antioxidation (oxidative stress and the prescence of free radicals), detoxification, and stimulation of glycolysis and glycogenesis.

A potentially important taurine role is in cell protection against hypoxia. (Canas PE (1992) Acta Physiol Pharmacol Ther Latinoam, 42(3), 133-7). Studies on renal cell cultures have shown that when taurine was administered during hypoxia, the cell damage was markedly reduced. It reduced the osmoregulatory deterioration during hypoxia and reoxygenation, so that calcium homeostasis was markedly improved. Furthermore, Ca²⁺ efflux during hypoxia as well as Ca²⁺

overload during reoxygenation was significantly reduced. The effect of taurine was partly comparable to the effect induced by Ca^{2+} channel blockers. One of the effects mainly responsible for cellular protection seems to be the taurine-induced acceleration of cellular growth processes in spite of hypoxia and reoxygenation. The spectrum of cytoprotective effects of taurine predisposes this substance to be a physiological protective agent responsible for cellular homeostasis or enantiostasis (Michalk DV *et al.*, (1996) *Adv Exp Med Biol*, 403, 223-32). In neuron damaging conditions it may constitute an important protective mechanism against excitotoxicity, e.g. ischemia (Saransaari P, Oja S S, (2000) *Amino Acids* 19 (3-4), 509-26).

Lastly, taurine, arginine and homocysteine are amino acids which have been shown to affect the risk factors of cardiovascular diseases in humans (Nittynen L, *et al.*, (1999) *Ann Med*, Oct;31(5), 318-26).

Hypoxia and tumour growth

Tumour growth is dependant on oxygen and nutrients supplied by the local tissue vasculature. Solid tumours are well known to be poorly oxygenated compared to normal tissue (In: Vaupel, P. W. *et al.*, (eds.) *Tumour Oxygenation* pp219-232: Gustav Fisher Verlag, 1995). Hypoxia (low cellular oxygen concentration, <1%) arises when tumour cells proliferate outside the diffusion zone of the local vascular supply. Tumours respond to hypoxia by producing hypoxia inducible factors (e.g. VEGF) that stimulate the growth of endothelial cells (the cells lining blood capillaries) from surrounding blood vessels (i.e. angiogenesis) (Weidner N, *et al.*, *N Engl J Med*, 1991, Jan 3, 324:1, 1-8). Blood flow in these tumour blood vessels is sluggish and irregular which results in less efficient oxygen delivery and propagates the hypoxic tendency of tumours (for review see, Brown J. M. *Mol Med Today* 6; 157-62, 2000). This hypoxia-induced angiogenic process allows tumour cells access to the host animal's circulatory system. Furthermore, the new blood vessels provide a gateway for tumor cells to enter the circulation and metastasize to distant sites (Folkman J, *J Natl Cancer Inst.*, 1990, Jan 3;82(1):4-6). In fact, the extent of neovascularity is strongly correlated with metastases in primary breast carcinoma, bladder cancer, prostate cancer, non-small cell lung cancer, cutaneous melanomas and uterine cervix carcinoma (reviewed in: Ferrara N, *Breast Cancer Res Treat*, 1995, 36:2, 127-37). These findings have led researchers to speculate that tumor vascularisation could be used as a diagnostic tool to predict the stage of the cancer (i.e. whether the cancer has metastasized or not and to what extent).

Hypoxia in Tumour Therapy

Carcinomas are known to have significant hypoxic fractions, e.g. 80% of the tumour for head and neck squamous cell carcinomas and 50% of the tumour for carcinoma of the uterine cervix (Van De Wiele, C *et al.*, (2001) *Nuclear Med*, 22, 945-947). The hypoxic areas are heterogeneous and are partly due to the different oxygen tensions present throughout the tumour. Hypoxic areas of tumours tend to escape radiation and chemotherapy. These areas are the furthest away from blood vessels and hence receive poor drug delivery. Hypoxic tumour cells also tend to be slow-proliferating and most chemotherapy drugs target rapidly dividing tumour cells only. This may be one of the reasons why hypoxia can induce relapse after treatment and the evolution of more aggressive and resistant tumours. Hypoxia increases the mutation rate of cells and results in mutated cell-types that are less susceptible to programmed cell death signals (such as p53). Overall, tumour hypoxia has emerged as a predictor of poor prognosis. Since hypoxia is an abnormal

condition that exists in all solid tumours, it could be used as a very specific target for anti-cancer therapies.

Therefore, a need exists to identify new markers and potential targets of the oxygen-regulated angiogenic pathway that are important in the development of cancers.

5 An ideal protein target for cancer immunotherapy should have a restricted expression profile in normal tissues and be over-expressed in tumours, such that the immune response will be targeted to tumour cells and not against other organs. In addition, the protein target should be exposed on the cell surface, where it will be accessible to therapeutic agents. Tumour antigens have been identified for a number of cancer types, by using techniques such as differential screening of
10 cDNA (Hubert, R.S., *et al.*, *Proc. Natl. Acad. Sci. USA* **96**, 14523-14528 (1999); Lucas, S., De Plaen, E. & Boon, T. *Int. J. Cancer* **87**, 55-60 (2000)), and the purification of cell-surface antigens that are recognised by tumour-specific antibodies (Catimel, B., *et al.*, *J. Biol. Chem.* **271**, 25664-25670 (1996)).

Angiogenesis

15 Under normal conditions, angiogenesis is necessary to facilitate wound healing, tissue repair, reproduction, growth and development. However, many disease states are also dependent upon this process. The process of wound healing is complex and represents a serious medical problem affecting a large number of individuals. Healing problems occur in dermal wounds, such as decubitus ulcers, severe burns, diabetic ulcers and eye lesions (including dry eye and corneal
20 ulcers) as well as surgical wounds and other wound-related pathologies. One important aspect of wound healing is the controlled migration of new cells from tissues surrounding the wound-site. This is in order to establish a proper population of cell types and correct tissue organization in the newly developing tissue. Hypoxia promoted increased vascular growth and is thus associated with tumour growth as described above. Additionally, excessive vascular growth is also known to
25 contribute to non-neoplastic disorders, such as diabetic retinopathy, asthma, macular degeneration, psoriasis and rheumatoid arthritis.

The present invention is based on the finding that SC6 plays a central role in the cellular response to hypoxia. Importantly, an increased expression of SC6 has been observed in both carcinoma cell lines and in tumour tissues. This finding was observed for a variety of cancer types
30 including, cervical, colon, renal, lung, uterine, breast and pancreatic cell carcinoma, Burkitt's lymphoma and myeloid and T-cell leukaemia, indicating that SC6 plays a role in the development of many cancer types.

The sequence for SC6, can be found in the GenBank database, held by the National Institute of Health (NIH) available at <http://www.ncbi.nlm.nih.gov/>- the amino acid sequence is
35 shown, under accession number NM_003034, in Figure 1 and the nucleic acid sequence is shown under accession number NM_003043, in Figure 2. SC6 had relatively low expression in most normal tissues investigated. However, elevated SC6 expression was observed in some tissue samples of cervical, colon, renal, lung and uterine cell carcinomas and in certain tumour cell lines e.g. Burkitt's lymphoma, myeloid and T-cell leukaemia, some breast cell carcinoma, renal and
40 pancreatic carcinoma cell lines, suggesting that SC6 may be a suitable target for cancer therapy and diagnosis. In addition, SC6 expression is increased under hypoxic conditions in human dermal microvascular endothelial (HDMEC) and renal cancer (RCC4) cell lines, indicating that it may respond to hypoxic factors.

The term hypoxia related condition includes conditions such as cancer, angiogenesis and angiogenesis dependant conditions. Cancer includes both solid tumours such as, cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, and hematopoietic tumours e.g. Burkitt's lymphoma or myeloid or T-cell leukaemia. Angiogenesis dependant conditions include cancer and non-neoplastic disorders, such as diabetic retinopathy, asthma, macular degeneration, psoriasis and rheumatoid arthritis.

The present invention contemplates the use of the full-length protein in the diagnosis, prognosis and treatment of hypoxia related conditions, in particular cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma or myeloid or T-cell leukaemia. The use of therapeutic antibodies raised against the extracellular portion of SC6 is specifically contemplated.

Thus, in a first aspect, the present invention relates to a method of screening for and/or diagnosis of hypoxia related conditions in a subject and or monitoring the effectiveness of carcinoma therapy, which comprises the step of detecting and/or quantifying in a biological sample obtained from said subject the amount of an SC6 polypeptide which

- a) comprises or consists of the amino acid sequence shown in Figure 1;
- b) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence shown in Figure 1; or
- c) is a fragment of a polypeptide as defined in a) or b) above, which is at least ten amino acids long.

Polypeptides for use in the present invention are in isolated or recombinant form, and may be fused to other moieties. In particular, fusions of the polypeptides of the present invention with localisation-reporter proteins such as the Green Fluorescent Protein (U.S. Patent Nos. 5,625,048, 5,777,079, 6,054,321 and 5,804,387) or the DsRed fluorescent protein (Matz, M. V., Fradkov, A. F., Labas, Y. A., Savitsky, A. P., Zaraisky, A. G., Markelov, M. L. & Lukyanov S. A. (1999). Fluorescent proteins from nonbioluminescent Anthozoa species. Nature Biotech. 17:969-973.) are specifically contemplated by the present invention. They are provided in substantially pure form, that is to say, they are free, to a substantial extent, from other proteins. Thus, a polypeptide for use in the present invention may be provided in a composition in which it is the predominant component present (i.e. it is present at a level of at least 50%; preferably at least 75%, at least 90%, or at least 95%; when determined on a weight/weight basis excluding solvents or carriers).

In order to more fully appreciate the present invention, polypeptides within the scope of a)-c) above will now be discussed in greater detail.

Polypeptides within the scope of a)

A polypeptide within the scope of a), may consist of the particular amino acid sequence given in Figure 1 or may have an additional N-terminal and/or an additional C-terminal amino acid sequence relative to the sequence given in Figure 1.

Additional N-terminal or C-terminal sequences may be provided for various reasons.

Techniques for providing such additional sequences are well known in the art. Additional sequences may be provided in order to alter the characteristics of a particular polypeptide. This can be useful in improving expression or regulation of expression in particular expression systems. For example, an additional sequence may provide some protection against proteolytic

cleavage. This has been done for the hormone Somatostatin by fusing it at its N-terminus to part of the β galactosidase enzyme (Itakwa *et al.*, *Science* 198: 105-63 (1977)).

Additional sequences can also be useful in altering the properties of a polypeptide to aid in identification or purification. For example, a fusion protein may be provided in which a polypeptide is linked to a moiety capable of being isolated by affinity chromatography. The moiety may be an antigen or an epitope and the affinity column may comprise immobilised antibodies or immobilised antibody fragments which bind to said antigen or epitope (desirably with a high degree of specificity). The fusion protein can usually be eluted from the column by addition of an appropriate buffer.

Additional N-terminal or C-terminal sequences may, however, be present simply as a result of a particular technique used to obtain a polypeptide of the present invention and need not provide any particular advantageous characteristic to the polypeptide of the present invention. Such polypeptide are within the scope of the present invention.

Whatever additional N-terminal or C-terminal sequence is present, it is preferred that the resultant polypeptide should exhibit the immunological activity of the polypeptide having the amino acid sequence shown in Figure 1.

Polypeptides within the scope of b)

Turning now to the polypeptides defined in b) above, it will be appreciated by the person skilled in the art that these polypeptides are variants of the polypeptide given in a) above, provided that such variants exhibit the immunological activity of the polypeptide having the amino acid sequence shown in Figure 1.

Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the protein's function.

The skilled person will appreciate that various changes can often be made to the amino acid sequence of a polypeptide which has a particular activity to produce variants (sometimes known as "muteins") having at least a proportion of said activity, and preferably having a substantial proportion of said activity. Such variants of the polypeptides described in a) above are within the scope of the present invention and are discussed in greater detail below. They include allelic and non-allelic variants.

An example of a variant for use in the present invention is a polypeptide as defined in a) above, apart from the substitution of one or more amino acids with one or more other amino acids. The skilled person is aware that various amino acids have similar properties. One or more such amino acids of a substance can often be substituted by one or more other such amino acids without eliminating a desired activity of that substance.

Thus, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions, it is preferred that glycine and alanine are used to substitute for one another (since they have relatively short side chains) and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic).

Other amino acids which can often be substituted for one another include:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Substitutions of this nature are often referred to as "conservative" or "semi-conservative" amino acid substitutions.

Amino acid deletions or insertions may also be made relative to the amino acid sequence given in a) above. Thus, for example, amino acids which do not have a substantial effect on the activity of the polypeptide, or at least which do not eliminate such activity, may be deleted. Such deletions can be advantageous since the overall length and the molecular weight of a polypeptide can be reduced whilst still retaining activity. This can enable the amount of polypeptide required for a particular purpose to be reduced - for example, dosage levels can be reduced.

Amino acid insertions relative to the sequence given in a) above can also be made. This may be done to alter the properties of a polypeptide of the present invention (e.g. to assist in identification, purification or expression, as explained above in relation to fusion proteins).

Amino acid changes relative to the sequence given in a) above can be made using any suitable technique e.g. by using site-directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551).

It should be appreciated that amino acid substitutions or insertions within the scope of the present invention can be made using naturally occurring or non-naturally occurring amino acids. Whether or not natural or synthetic amino acids are used, it is preferred that only L- amino acids are present.

Whatever amino acid changes are made (whether by means of substitution, insertion or deletion), preferred polypeptides of the present invention have at least 50% sequence identity with a polypeptide as defined in a) above, more preferably the degree of sequence identity is at least 75%. Yet more preferably the degree of sequence identity is at least 80% or at least 85%. Sequence identities of at least 90% or at least 95% are most preferred.

The percent identity of two amino acid sequences or of two nucleic acid sequences is determined by aligning the sequences for optimal comparison purposes (e.g., gaps can be introduced in the first sequence for best alignment with the sequence) and comparing the amino acid residues or nucleotides at corresponding positions. The "best alignment" is an alignment of two sequences which results in the highest percent identity. The percent identity is determined by the number of identical amino acid residues or nucleotides in the sequences being compared (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm known to those of skill in the art. An example of a mathematical algorithm for comparing two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The NBLAST and XBLAST programs of Altschul, et al. (1990) *J. Mol. Biol.* 215:403-410 have incorporated such an algorithm. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences

homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilised as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (*Id.*). When utilising BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

Another example of a mathematical algorithm utilised for the comparison of sequences is the algorithm of Myers & Miller, CABIOS (1989). The ALIGN program (version 2.0) which is part of the CGC sequence alignment software package has incorporated such an algorithm. Other algorithms for sequence analysis known in the art include ADVANCE and ADAM as described in Torellis & Robotti (1994) *Comput. Appl. Biosci.*, 10 :3-5; and FASTA described in Pearson & Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-8. Within FASTA, ktup is a control option that sets the sensitivity and speed of the search.

Where high degrees of sequence identity are present there will be relatively few differences in amino acid sequence. Thus for example they may be less than 20, less than 10, or even less than 5 differences.

Polypeptides within the scope of c)

As discussed *supra*, it is often advantageous to reduce the length of a polypeptide, provided that the resultant reduced length polypeptide still has a desired activity or can give rise to useful antibodies. Feature c) for use in the present invention therefore covers fragments of polypeptides a) or b) above.

The skilled person can determine whether or not a particular fragment has activity using the techniques disclosed above. Preferred fragments are at least 10 amino acids long. They may be at least 20, at least 50 or at least 100 amino acids long.

As will be discussed below, the polypeptides for use in the present invention will find use in a therapeutic approach to hypoxia related conditions, particularly for cancer. The skilled person will appreciate that for the preparation of one or more polypeptides for use in the invention, the preferred approach will be based on recombinant DNA techniques.

The present invention also provides a method of screening for and/or diagnosis of hypoxia related conditions in a subject and or monitoring the effectiveness of carcinoma therapy, which comprises the step of detecting and/or quantifying in a biological sample obtained from said subject the amount of an isolated or recombinant DNA nucleic acid sequence which

- a) comprises or consists of the DNA sequence shown in Figure 2 or its RNA equivalent;
- b) has a sequence which is complementary to the sequences of a);
- c) has a sequence which codes for the same polypeptide, as the sequences of a) or b);
- d) has a sequence which shows substantial identity with any of those of a), b) and c); or
- e) a sequence which codes for a derivative or fragment of an amino acid molecule shown in Figure 1.

These nucleic acid molecules are now discussed in greater detail.

It is preferred if sequences which show substantial identity with any of those of a), b) and c) have e.g. at least 50%, at least 75%, at least 80%, at least 85% or at least 90% or 95% sequence identity.

The polypeptides of the present invention can be coded for by a large variety of nucleic acid molecules, taking into account the well-known degeneracy of the genetic code. All of these molecules are within the scope of the present invention. They can be inserted into vectors and cloned to provide large amounts of DNA or RNA for further study. Suitable vectors may be introduced into host cells to enable the expression of polypeptides of the present invention using techniques known to the person skilled in the art.

The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may be in isolated, recombinant or chemically synthetic form.

Techniques for cloning, expressing and purifying proteins and polypeptides are well known to the skilled person. DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2nd Edition*, Cold Spring Harbour Laboratory Press (1989); in Old & Primrose *Principles of Gene Manipulation* 5th Edition, Blackwell Scientific Publications (1994); and in Stryer [*Biochemistry* 4th Edition, W H Freeman and Company (1995)]. Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

Normally the DNA construct will be inserted into a vector, which may be of phage or plasmid origin. Expression of the protein is achieved by the transformation or transfection of the vector into a host cell which may be of eukaryotic or prokaryotic origin. Such vectors and suitable host cells form further aspects of the present invention.

Knowledge of the nucleic acid structure can be used to raise antibodies and for gene therapy. Techniques for this are well-known by those skilled in the art.

By using appropriate expression systems, polypeptides for use in the present invention may be expressed in glycosylated or non-glycosylated form. Non-glycosylated forms can be produced by expression in prokaryotic hosts, such as *E. coli*.

Polypeptides comprising N-terminal methionine may be produced using certain expression systems, whilst in others the mature polypeptide will lack this residue.

Preferred techniques for cloning, expressing and purifying a substance of the present invention are summarised below:

Polypeptides may be prepared natively or under denaturing conditions and then subsequently refolded. Baculoviral expression vectors include secretory plasmids (such as pACGP67 from Pharmingen), which may have an epitope tag sequence cloned in frame (e.g. myc, V5 or His) to aid detection and allow for subsequent purification of the protein. Mammalian expression vectors may include pCDNA3 and pSecTag (both Invitrogen), and pREP9 and pCEP4 (Invitrogen). *E. coli* systems include the pBad series (His tagged - Invitrogen) or pGex series (Pharmacia).

In addition to nucleic acid molecules coding for polypeptides for use in the present invention, referred to herein as "coding" nucleic acid molecules, the present invention also includes nucleic acid molecules complementary thereto. Thus, for example, both strands of a double stranded nucleic acid molecule are included within the scope of the present invention (whether or not they are associated with one another). Also included are mRNA molecules and complementary DNA Molecules (e.g. cDNA molecules).

Nucleic acid molecules that can hybridise to any of the nucleic acid molecules discussed above are also covered by the present invention. Such nucleic acid molecules are referred to herein as "hybridising" nucleic acid molecules. Hybridising nucleic acid molecules can be useful as probes or primers, for example.

Desirably such hybridising molecules are at least 10 nucleotides in length and preferably are at least 25 or at least 50 nucleotides in length. The hybridising nucleic acid molecules preferably hybridise to nucleic acids within the scope of a), b), c), d), or e) above specifically.

Desirably the hybridising molecules will hybridise to such molecules under stringent hybridisation conditions. One example of stringent hybridisation conditions is where attempted hybridisation is carried out at a temperature of from about 35°C to about 65°C using a salt solution, which is about 0.9 molar. However, the skilled person will be able to vary such conditions as appropriate in order to take into account variables such as probe length, base composition, type of ions present, etc.

Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

Typically primers will be at least five nucleotides long and will generally be at least ten nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases, primers of at least thirty or at least thirty-five nucleotides in length may be used.

As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to provide a longer sequence.

In addition to being used as primers and/or probes, hybridising nucleic acid molecules of the present invention can be used as anti-sense molecules to alter the expression of substances of the present invention by binding to complementary nucleic acid molecules. This technique can be used in anti-sense therapy.

A hybridising nucleic acid molecule of the present invention may have a high degree of sequence identity along its length with a nucleic acid molecule within the scope of (a)-(e) above (e.g. at least 50%, at least 75%, at least 80%, at least 85% or at least 90% or 95% sequence identity). As will be appreciated by the skilled person, the higher the sequence identity a given single stranded nucleic acid molecule has with another nucleic acid molecule, the greater the likelihood that it will hybridise to a nucleic acid molecule which is complementary to that other nucleic acid molecule under appropriate conditions.

In view of the foregoing description the skilled person will appreciate that a large number of nucleic acids are within the scope of the present invention. Unless the context indicates otherwise, nucleic acid molecules of the present invention may have one or more of the following characteristics:

- 1) they may be DNA or RNA;
- 2) they may be single or double stranded;
- 3) they may be provided in recombinant form i.e. covalently linked to a 5' and/or a 3' flanking sequence to provide a molecule which does not occur in nature;
- 5 4) they may be provided without 5' and/or 3' flanking sequences which normally occur in nature;
- 5) they may be provided in substantially pure form. Thus they may be provided in a form which is substantially free from contaminating proteins and/or from other nucleic acids; and
- 6) they may be provided with introns or without introns (e.g. as cDNA).

10 A convenient means for such detection/quantifying will involve the use of antibodies. Thus, the polypeptides of the invention also find use in raising antibodies. Thus, in another aspect, the present invention provides antibodies, which bind to a polypeptide of the present invention or to a fragment of such a polypeptide. Preferred antibodies bind specifically to polypeptides of the present invention so that they can be used to purify and/or inhibit the activity of such polypeptides. The

15 antibodies may be monoclonal or polyclonal.

Thus, the SC6 polypeptide, its fragments or other derivatives, or analogues thereof, may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments,

20 fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA)

25 or subclass of immunoglobulin molecule.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies, which recognize a specific domain of a polypeptide of the invention, one may assay generated hybridomas for a product which binds to a polypeptide fragment containing such

30 domain. For selection of an antibody that specifically binds a first polypeptide homologue but which does not specifically bind to (or binds less avidly to) a second polypeptide homologue, one can select on the basis of positive binding to the first polypeptide homologue and a lack of binding to (or reduced binding to) the second polypeptide homologue.

For preparation of monoclonal antibodies (mAbs) directed toward a polypeptide of the invention or fragment or analogue thereof, any technique which provides for the production of

35 antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al.,

40 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated in vitro or in vivo. In an additional embodiment of the invention, monoclonal antibodies can be produced in

germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

5 The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (e.g., human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., Cabilly et al., U.S. Patent No. 4,816,567; and Boss et al., U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Patent No. 5,585,089, which is incorporated herein by reference in its entirety.)

10 Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al., 1988, Science 240:1041-1043; Liu et al., 1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Canc. Res. 47:999-1005; 20 Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi et al., 1986, Bio/Techniques 4:214; U.S. Patent 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060.

25 Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human 30 immunoglobulin transgenes harboured by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., 35 U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

40 Completely human antibodies, which recognize a selected epitope, can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespersen et al. (1994) Bio/technology 12:899-903).

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labelled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988).

The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., 1991, *EMBO J.* 10:3655-3659.

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain

constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression
5 vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in
10 high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific
15 compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details for generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 1986, 121:210.

The invention provides functionally active fragments, derivatives or analogues of the
20 anti-polypeptide immunoglobulin molecules. Functionally active means that the fragment, derivative or analogue is able to elicit anti-anti-idiotypic antibodies (i.e., tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analogue is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that
25 are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')₂ fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be
30 generated by known techniques. F(ab')₂ fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')₂ fragments. The invention also provides heavy chain and light chain dimmers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies
35 (SCAs) (e.g., as described in U.S. Patent 4,946,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv
40 fragments in *E. coli* may be used (Skerra et al., 1988, *Science* 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an

amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life in vivo, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogues and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogues of the immunoglobulins include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analogue or derivative may contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the polypeptides of the invention, e.g., for imaging or radioimaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc. and for radiotherapy.

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression technique.

Recombinant expression of antibodies, or fragments, derivatives or analogues thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridisable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by

screening antibody libraries (See, e.g., Clackson et al., 1991, Nature 352:624; Hane et al., 1997 Proc. Natl. Acad. Sci. USA 94:4937).

Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, in vitro site directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem. 253:6551), PCT based methods, etc.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, e.g., humanized antibodies.

Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the protein of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing an antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and Ausubel et al. (eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., 198, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding

sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harbouring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (e.g., an adenovirus expression system) may be utilized.

As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (e.g., neomycin or hygromycin), and selecting for

expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

5 The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody
10 will also increase (Crouse et al., 1983, Mol. Cell. Biol. 3:257).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which
15 encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, Nature 322:52; Kohler, 1980, Proc. Natl. Acad. Sci. USA 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once the antibody molecule of the invention has been recombinantly expressed, it may be
20 purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g., ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific
25 for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag
30 serves as a matrix-binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

In a preferred embodiment, antibodies of the invention or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the
35 efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be
40 conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine,

dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I , ^{131}I , ^{111}In and ^{99}Tc .

Antibodies of the invention or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

Another aspect of the invention provides methods of screening for agents that modulate (e.g., upregulate or downregulate) a characteristic of, e.g., the expression or the transporter or binding activity, of a polypeptide of the invention.

The invention provides methods for identifying agents (e.g., chemical compounds, proteins, or peptides) that bind to a polypeptide of the invention or have a modulatory effect (e.g. stimulatory, inhibitory, up-regulation, downregulation) effect on the expression or activity of a polypeptide of the invention. Examples of candidate agents, include, but are not limited to, nucleic acids (e.g., DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, agonists, antagonists, small molecules and other drugs. Agents can be obtained using any of the numerous suitable approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer

or small molecule libraries of compounds (Lam, 1997, Anticancer Drug Des. 12:145; U.S. 5,738,996; and U.S. 5,807,683, each of which is incorporated herein in its entirety by reference).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., 1993, Proc. Natl. Acad. Sci. USA 90:6909; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al., 1994, J. Med. Chem. 37:2678; Cho et al., 1993, Science 261:1303; Carrell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al., 1994, Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al., 1994, J. Med. Chem. 37:1233, each of which is incorporated herein in its entirety by reference.

Libraries of compounds may be presented, e.g., presented in solution (e.g., Houghten, 1992, Bio/Techniques 13:412-421), or on beads (Lam, 1991, Nature 354:82-84), chips (Fodor, 1993, Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, Proc. Natl. Acad. Sci. USA 89:1865-1869) or phage (Scott and Smith, 1990, Science 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici, 1991, J. Mol. Biol. 222:301-310), each of which is incorporated herein in its entirety by reference.

In one embodiment, agents that interact with (i.e., bind to) a polypeptide of the invention are identified in a cell-based assay system. In accordance with this embodiment, cells expressing polypeptide of the invention are contacted with a candidate agent or a control agent and the ability of the candidate agent to interact with the polypeptide is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate agents. The cell, for example, can be of prokaryotic origin (e.g., *E. coli*) or eukaryotic origin (e.g., yeast or mammalian). Further, the cells can express the polypeptide of the invention endogenously or be genetically engineered to express the polypeptide. In some embodiments, polypeptide of the invention or the candidate agent is labelled, for example with a radioactive label (such as ^{32}P , ^{35}S or ^{125}I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between a polypeptide and a candidate agent. The ability of the candidate agent to interact directly or indirectly with the polypeptide of the invention can be determined by methods known to those of skill in the art. For example, the interaction between a candidate agent and a polypeptide can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

In another embodiment, agents that interact with (i.e., bind to) a polypeptide of the invention are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant polypeptide of the invention or fragment thereof is contacted with a candidate agent or a control agent and the ability of the candidate agent to interact with the polypeptide is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate agents. Preferably, the polypeptide is first immobilized, by, for example, contacting the polypeptide with an immobilized antibody which specifically recognizes and binds it, or by contacting a purified preparation of polypeptide with a surface designed to bind proteins. The polypeptide may be partially or completely purified (e.g., partially or completely free of other polypeptides) or part of a cell lysate. Further, the polypeptide may be a fusion protein comprising the polypeptide of the invention or a biologically active portion thereof and a domain such as glutathionine-S-transferase. Alternatively, the polypeptide can be biotinylated using techniques well known to those of skill in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL). The

ability of the candidate agent to interact with the polypeptide can be determined by methods known to those of skill in the art.

In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of the SC6 polypeptide or is responsible for the post-translational modification of the polypeptide. In a primary screen, a plurality (*e.g.*, a library) of agents are contacted with cells that naturally or recombinantly express: (i) a polypeptide of the invention; and (ii) a protein that is responsible for processing of the polypeptide in order to identify compounds that modulate the production, degradation, or post-translational modification of the polypeptide. If desired, agents identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing the specific polypeptide of interest. The ability of the candidate agent to modulate the production, degradation or post-translational modification of a polypeptide can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

In another embodiment, agents that competitively interact with (*i.e.*, bind to) an SC6 polypeptide are identified in a competitive binding assay. In accordance with this embodiment, cells expressing the polypeptide are contacted with a candidate agent and as agent known to interact with the polypeptide; the ability of the candidate agent to competitively interact with the polypeptide is then determined. Alternatively, agents that competitively interact with (*i.e.*, bind to) a polypeptide are identified in a cell-free assay system by contacting the polypeptide with a candidate agent and an agent known to interact with the polypeptide. As stated above, the ability of the candidate agent to interact with an SC6 polypeptide can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (*e.g.*, a library) of candidate agents.

In another embodiment, agents that modulate (*i.e.*, upregulate or downregulate) the expression of a polypeptide of the invention are identified by contacting cells (*e.g.*, cells of prokaryotic origin or eukaryotic origin) expressing the polypeptide with a candidate agent or a control agent (*e.g.*, phosphate buffered saline (PBS)) and determining the expression of the polypeptide or mRNA encoding the polypeptide. The level of expression of a selected polypeptide or mRNA encoding polypeptide in the presence of the candidate agent is compared to the level of expression of the polypeptide or mRNA encoding the polypeptide in the absence of the candidate agent (*e.g.*, in the presence of a control agent). The candidate agent can then be identified as a modulator of the expression of the polypeptide based on this comparison. For example, when expression of the polypeptide or mRNA encoding the polypeptide is significantly greater in the presence of the candidate agent than in its absence, the candidate agent is identified as a stimulator of expression of the polypeptide or mRNA encoding the polypeptide. Alternatively, when expression of the polypeptide or mRNA encoding the polypeptide is significantly less in the presence of the candidate agent than in its absence, the candidate agent is identified as an inhibitor of the expression of the polypeptide or mRNA encoding the polypeptide. The level of expression of a polypeptide of the invention or the mRNA that encodes it can be determined by methods known to those of skill in the art based on the present description. For example, mRNA expression

can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

5 In another embodiment, agents that modulate the activity of a polypeptide are identified by contacting a preparation containing the polypeptide, or cells (*e.g.*, prokaryotic or eukaryotic cells) expressing the polypeptide with a candidate agent or a control agent and determining the ability of the candidate agent to modulate (*e.g.*, stimulate or inhibit) the activity of polypeptide. The activity of a polypeptide can be assessed by detecting its effect on a "downstream effector" for example, but without limitation, induction of a cellular signal transduction pathway of the polypeptide (*e.g.*, intracellular Ca^{2+} , diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of the target
10 on a suitable substrate, detecting the induction of a reporter gene (*e.g.*, a regulatory element that is responsive to a polypeptide of the invention and is operably linked to a nucleic acid encoding a detectable marker, *e.g.*, luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation as the case may be, based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, *e.g.*, U.S. Patent
15 No. 5,401,639, which is incorporated in its entirety herein by reference). The candidate agent can then be identified as a modulator of the activity of a polypeptide of the invention by comparing the effects of the candidate agent to the control agent. Suitable control agents include phosphate buffered saline (PBS) and normal saline (NS).

20 In another embodiment, agents that modulate (*i.e.*, upregulate or downregulate) the expression, activity or both the expression and activity of a polypeptide of the invention are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. In accordance with this embodiment, the candidate agent or a control agent is administered (*e.g.*, orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or
25 both expression and activity of the polypeptide is determined. Changes in the expression of a polypeptide can be assessed by any suitable method described above, based on the present description.

30 In yet another embodiment, a polypeptide or the invention is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with the polypeptide (see, *e.g.*, U.S. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and WO 94/10300). As those skilled in the art will appreciate, such binding proteins are also likely to be involved in the propagation of signals by the polypeptides of the invention as, for example, upstream or downstream elements of a signalling
35 pathway involving the polypeptides of the invention.

This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

40 As used herein "active agent" refers to the polypeptides of the invention and nucleic acid molecules encoding the polypeptides, antibodies against the polypeptides and agents *e.g.* small molecules, which modulate the expression of the polypeptides of the invention.

As discussed herein, active agents of the invention find use in the treatment or prophylaxis of hypoxia related conditions *e.g.* cancer. Thus, in another aspect, the present invention provides a pharmaceutical composition comprising at least one active agent, optionally together with one or

more pharmaceutically acceptable excipients, carriers or diluents. In another aspect of the invention the pharmaceutical composition is for use as a vaccine and so any additional components will be acceptable for vaccine use. In addition, the skilled person will appreciate that one or more suitable adjuvants may be added to such vaccine preparations.

5 The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. This pharmaceutical composition may be in any suitable form, (depending upon the desired method of administering it to a patient).

10 It may be provided in unit dosage form, will generally be provided in a sealed container and may be provided as part of a kit. Such a kit would normally (although not necessarily) include instructions for use. It may include a plurality of said unit dosage forms.

15 The pharmaceutical composition may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal, sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such compositions may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with the carrier(s) or excipient(s) under sterile conditions.

Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions).

20 Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof.

Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc.

25 For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions, oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

30 Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis as generally described in *Pharmaceutical Research*, 3(6):318 (1986).

35 Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils. For infections of the eye or other external tissues, for example mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base. Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. Pharmaceutical compositions adapted for topical administration in the mouth include 40 lozenges, pastilles and mouth washes.

Pharmaceutical compositions adapted for rectal administration may be presented as suppositories or enemas.

Pharmaceutical compositions adapted for nasal administration wherein the carrier is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the carrier is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient.

Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists, which may be generated by means of various types of metered dose pressurised aerosols, nebulisers or insufflators.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

The pharmaceutical compositions may contain preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colourants, odourants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention.

Dosages of the substance of the present invention can vary between wide limits, depending upon the disease or disorder to be treated, the age and condition of the individual to be treated, etc. and a physician will ultimately determine appropriate dosages to be used. This dosage may be repeated as often as appropriate. If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice.

In another aspect, the present invention provides a method for the prophylaxis and/or treatment of hypoxia related conditions. In a preferred aspect, it provides a method for the prophylaxis and/or treatment of cancer such as cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma or myeloid or T-cell leukaemia, in a subject, which comprises administering to said subject a therapeutically effective amount of at least one polypeptide or fragment thereof, nucleic acid molecule or antibody or other active agent of the invention.

In another aspect, the present invention provides the use of at least one polypeptide or fragment thereof, nucleic acid molecule or antibody or other active agent of the invention in the preparation of a medicament for use in the prophylaxis and/or treatment of hypoxia related conditions. A preferred aspect is for the use of at least one polypeptide or fragment thereof, nucleic acid molecule or antibody or other active agent of the invention in the preparation of a medicament

for use in the prophylaxis and/or treatment of cancer such as cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma, or myeloid or T-cell leukaemia. In particular, the preparation of vaccines and/or compositions comprising or consisting of antibodies is a preferred embodiment of this aspect of the invention.

In view of the importance of SC6 in cancer the following form additional aspects of the present invention:

- i) a method for monitoring/assessing cancer treatment, such as cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma or myeloid or T-cell leukaemia treatment in a patient, which comprises the step of determining the presence or absence and/or quantifying at least one SC6 polypeptide in a biological sample obtained from said patient;
- ii) a method for the identification of hypoxic cancer cells, such as hypoxic cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma or myeloid or T-cell leukaemia cells, in a biological sample obtained from a subject, which comprises the step of determining the presence or absence and/or quantifying at least one SC6 polypeptide.

In the context of the present invention, the biological sample can be obtained from any source, such as a serum sample or a tissue sample, e.g. breast tissue.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

The invention will now be described with reference to the following examples, which should not in any way be construed as limiting the scope of the present invention. The examples refer to the figures in which:

Figure 1: shows the amino acid sequence of SC6 (accession number NP_003034 in the GenBank database, held by the National Institute of Health (NIH) (available at <http://www.ncbi.nlm.nih.gov/>)).

Figure 2: shows the nucleotide sequence of SC6 (accession number NM_003043 in the GenBank database, held by the National Institute of Health (NIH) (available at <http://www.ncbi.nlm.nih.gov/>)).

Figure 3: shows the tissue distribution of SC6 mRNA. Levels of SC6 mRNA were quantified in a panel of normal tissues by real time RT-PCR. mRNA levels are expressed as the number of copies ng^{-1} cDNA.

Figure 4: shows the expression of SC6 mRNA in matched tumour and control tissue from the breast, cervix, colon, kidney, lung, thymus and uterus. Levels of SC6 mRNA were quantified by real time RT-PCR. mRNA levels are expressed as the number of copies ng^{-1} cDNA.

Figure 5: shows the expression of mRNA encoding SC6 in various cancer cell lines. The panel of human cell lines investigated are as follows: Burkitt's lymphoma (daudi and raji); myeloid leukaemia (HL-60); T-cell leukaemia (Jurkat); CD34; human osteosarcoma (MG63); 453; 468; breast carcinoma (BT20, BT474, CAL51, DU4475 and T47D); renal carcinoma (Wilm); transformed normal renal cell line (293); transformed normal fibroblast cell line (MRC-5); uterine sarcoma (MES-SA); cervical carcinoma (HeLa S3); pancreatic

carcinoma (Mia Paca, Panc 1, PC3, PC3M); human dermal microvascular endothelial cells (HDMECQ and HDMECQ-VEGF). Levels of SC6 mRNA were quantified by real time RT-PCR. mRNA levels are expressed as the number of copies ng^{-1} cDNA.

Figure 6: shows the expression of SC6 mRNA in human dermal microvascular endothelial cells (HDMEC) under normoxic and hypoxic conditions. Levels of SC6 mRNA were quantified by real time RT-PCR. mRNA levels are expressed as the number of copies ng^{-1} cDNA.

Figure 7: shows the expression of SC6 mRNA in renal cell carcinoma cells (RCC4), which have been stably transfected with a vector containing the VHL gene or a control vector (wild type). Levels of SC6 mRNA were quantified under normoxic and hypoxic conditions. by real time RT-PCR. mRNA levels are expressed as the number of copies ng^{-1} cDNA.

Example 1: Expression of SC6 mRNA in human tissues

Real time quantitative RT-PCR was used (Heid, C.A., Stevens, J., Livak, K.J. & Williams, P.M. *Genome Res.* 6, 986-994 (1996); Morrison, T.B., Weis, J.J. & Wittwer, C.T. *Biotechniques* 24, 954-958 (1998)) to analyse the distribution of SC6 mRNA in normal human tissues (Figure 3) and in matched clinical normal and tumour cancer tissues (Figure 4), and in tumour cell lines (Figure 5).

Quantification of SC6 mRNA by RT-PCR

RT-PCR was used to quantitatively measure SC6 expression in normal human tissue mRNAs (Clontech), and in matched cancer tissues and normal tissue (Clontech).

The primers used for PCR were as follows:

sense, 5' atcggtatgctcctcgttgtaa- 3'

antisense, 5' agttggaggagctgatggtgat - 3'

Reactions containing 5ng cDNA, prepared using Superscript first strand synthesis for RT-PCR kit (Life Technologies), SYBR green sequence detection reagents (PE Biosystems) and sense and antisense primers were assayed on an ABI7700 sequence detection system (PE Biosystems). The PCR conditions were 1 cycle at 95°C for 10min followed by 40 cycles of 95°C for 30 sec and 65°C for 1min. The accumulation of PCR product was measured in real time as the increase in SYBR green fluorescence, and the data were analysed using the Sequence Detector program v1.6.3 (PE Biosystems). Standard curves relating initial template copy number to fluorescence and amplification cycle were generated using the amplified PCR product as a template, and were used to calculate SC6 copy number in each sample. The expression of SC6 mRNA is shown as a relative expression level of mRNA (copy number ng^{-1} cDNA).

The expression of SC6 mRNA was low in most normal tissues, with the highest levels of expression found in tonsil, trachea and normal mammary tissues (Figure 3).

The level of SC6 mRNA was quantified in matched normal and tumour tissue from breast, cervix, colon, kidney, lung, thymus and uterus samples (Figure 4). These samples were obtained from BD Biosciences Clontech, 1020 East Meadow Circle, Palo Alto, CA 94303.

We found SC6 mRNA expression was increased in cervical (1/1), colon (2/3), kidney (1/1), lung (2/1), and uterine (1/1) cell carcinoma relative to the corresponding matched control samples.

The expression of SC6 mRNA was further determined in a number of human tumour cell lines (Figure 5). The following list of human cell lines were investigated: Burkitt's lymphoma (daudi and raji); myeloid leukaemia (HL-60); T-cell leukaemia (Jurkat); CD34 (B-cells); human osteosarcoma (MG63); breast carcinoma (MDA-MB-453; MDA-MB-468; BT20, BT474, CAL51, DU4475 and T47D); renal carcinoma (Wilm); transformed normal renal cell line (293); transformed normal fibroblast cell line (MRC-5); uterine sarcoma (MES-SA); cervical carcinoma (HeLa S3); pancreatic carcinoma (Mia Paca, Panc 1, PC3, PC3M); human dermal microvascular endothelial cells (HDMECQ and HDMECQ-VEGF). These cell lines were obtained from the following suppliers: American Type Culture Collection (ATCC), Manassas, USA; European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK and BioWhittaker House, Wokingham, Berkshire, UK and were grown in media according to the suppliers instructions.

We found that SC6 mRNA levels were increased in Burkitt's lymphoma, myeloid and T-cell leukaemia, some breast cell carcinoma, renal and pancreatic carcinoma cell lines.

Example 2: Expression of SC6 mRNA under hypoxic conditions

Growth of primary endothelial cells under normoxic and hypoxic conditions

HDMEC cells were treated by exposing them to either normoxic or hypoxic (1% O₂) conditions for 16h (methodology for cell culture as in Ratcliffe P *et al.*, *Nature*, 1999, 399, 271-275). After exposure, the cells were harvested for mRNA preparation.

Gene Expression Microarray (GEM) analysis

A detailed analysis of differential gene expression in HDMEC cells grown under normoxic (control) or hypoxic (treated) conditions for 4, 8 and 16h was performed using the GEMTM microarray services offered by Incyte Genomics, Ltd. Botanic House, 100 Hills Road, Cambridge CB2 1FF, United Kingdom. In brief, researchers send pairs of polyA⁺ mRNAs that correspond to the key comparisons under investigation (in this instance hypoxia versus normoxia). Preparation of mRNA was performed using the Incyte protocol. Incyte, then prepare labeled probes and conduct a competitive hybridization reaction with the probes on one of their standard human microarrays. The human gene expression microarray (GEM) contains 5,686 annotated genes/clusters and 2,949 unannotated genes/clusters mapped to the Unigene database. The array is then scanned and a data file is transmitted back to the customer for further analysis.

GEM analysis of primary endothelial cells showed that the gene for SC6 was increased under hypoxia.

Table 1. Fold change of SC6 from GEM analysis using 10,000cDNA Incyte arrays

Hypoxia (h)	4	8	16
Fold increase in SC6 expression compared to cells under normoxia	2.4	2.8	6.1

Quantification of SC6 mRNA by RT-PCR

To confirm the GEM analysis results, we used real time quantitative RT-PCR (as described in Example 1) to analyse the expression of SC6 mRNA in primary endothelial cell cultures (Figure 6) and in renal carcinoma cell lines (Figure 7) under normoxic and hypoxic conditions.

The expression of SC6 mRNA was quantified in primary human dermal microvascular (HDMEC) endothelial cells after 4, 8 and 16 h of exposure to normoxic or hypoxic conditions (Figure 6). In agreement with the results from GEM analysis, we found a dramatic increase in SC6 mRNA levels after 16h of hypoxia relative to the normoxic sample.

The expression of SC6 mRNA was also measured in RCC4 cells, a renal cell carcinoma cell line that is deficient in the Von Hippel-Lindau (VHL) gene. These cells are unable to degrade hypoxia inducible factor (HIF), a transcription initiator protein, since this requires the formation of a VHL-HIF complex. Thus, the cells continuously produce factors that stimulate angiogenesis under both hypoxic and normoxic conditions (i.e. functional HIF can always be assembled). Stable transfection of the wild-type VHL gene back into the RCC4 cell line restores the cells ability to regulate the angiogenic response under hypoxic or normoxic conditions. Hence under normoxic conditions, the cells no longer produce hypoxic/ angiogenic stimulating factors.

The RCC4 cell line was transfected with either a VHL containing vector (RCC4 with VHL) or with an empty vector (RCC4). The two cell phenotypes were then cultured under hypoxic or normoxic conditions for 16h. SC6 mRNA was quantified in both cell phenotypes under both hypoxic and normoxic conditions (Figure 7).

The presence of hypoxia was associated with a large increase in the expression of SC6 mRNA in both cell phenotypes indicating that SC6 expression is induced by hypoxic factors. In addition, an increase in SC6 expression was observed in the cell phenotype with VHL expression (RCC4 with VHL) compared to the RCC4 cell phenotype under normoxic conditions possibly indicating that the VHL protein may regulate the induction of SC6 expression. Since no SC6 mRNA expression was observed in the RCC4 cell phenotype under normoxic conditions, it seems unlikely that SC6 expression is regulated by a HIF-mediated pathway.

In summary, SC6 shows a low expression profile in normal tissues but is elevated in certain tumour tissues and tumorigenic cell lines e.g. cervical, colon, renal, lung, uterine, breast and pancreatic cell carcinoma in addition to Burkitt's lymphoma and myeloid and T-cell leukaemia. In particular, SC6 is elevated under hypoxic conditions indicating that it may be a cellular marker of hypoxic tumours.

SC6 up-regulation may serve to increase taurine levels within the hypoxic tumour cell and hence protect the tumour cell from cell damage due to the low oxygen concentrations. Further, this would aid the proliferation and motility of the tumour cells. Thus, SC6 may have a use in the diagnosis or treatment of cancer.

The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The contents of each reference, patent and patent application cited in this application are hereby incorporated by reference in its entirety.

When a reference is made herein to a method of treating or preventing a disease or condition using a particular agent or combination of agents, it is to be understood that such a reference is intended to include the use of that agent or combination of agents in the preparation of a medicament for the treatment or prevention of the disease or condition.

Preferred features of each aspect of the invention are as for each of the other aspects *mutatis mutandis*.

CLAIMS

1. A method of screening for and/or diagnosis of hypoxia related conditions in a subject and or monitoring the effectiveness of therapy, which comprises the step of detecting and/or quantifying in a biological sample obtained from said subject

(i) an SC6 polypeptide which

- a) comprises or consists of the amino acid sequence shown in Figure 1;
- b) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence shown in Figure 1; or
- c) is a fragment of a polypeptide as defined in a) or b) above, which is at least ten amino acids long;

(ii) a nucleic acid molecule which

- a) comprises or consists of the DNA sequence shown in Figure 2 or its RNA equivalent;
- b) has a sequence which is complementary to the sequences of a);
- c) has a sequence which codes for the same polypeptide as the sequences of a) or b);
- d) has a sequence which shows substantial identity with any of those of a), b) and c); or
- e) a sequence which codes for a derivative or fragment of a polypeptide as shown in

Figure 1.

2. The method according to claim 1 wherein the MW of the SC6 polypeptide is about 69.9 kDa.

3. The method according to claim 1 wherein the MW of the SC6 polypeptide is within 10% of 69.9 kDa.

4. An antibody that specifically binds to one or more SC6 polypeptides as defined in claim 1(i).

5. The method according to claim 1 wherein the polypeptide is detected and/ or quantified using an antibody that specifically binds to one or more SC6 polypeptides as defined in claim 1(i).

6. An antibody according to claim 4 or the method of claim 5 wherein the antibody is monoclonal, polyclonal, chimeric, humanised or bispecific, or is conjugated to a therapeutic moiety, second antibody or a fragment thereof, a cytotoxic agent or cytokine.

7. A method for the prophylaxis and/ or treatment of hypoxia related conditions in a subject, which comprises administering to said subject a therapeutically effective amount of

- (i) at least one SC6 polypeptide as defined in claim 1(i), 2, or 3,
- (ii) a nucleic acid molecule as defined in claim 1(ii), or
- (iii) an antibody as defined in claim 4 or 6.

8. The use of

- (i) at least one SC6 polypeptide as defined in claim 1(i), 2, or 3,

(ii) a nucleic acid molecule as defined in claim 1(ii), or
 (iii) an antibody as defined in claim 4 or 6, in the preparation of a medicament for use in the prophylaxis and/ or treatment of hypoxia related conditions.

- 5 9. (i) at least one SC6 polypeptide as defined in claim 1(i), 2, or 3,
 (ii) a nucleic acid molecule as defined in claim 1(ii), or
 (iii) an antibody as defined in claim 4 or 6
 for use in the prophylaxis and/ or treatment of hypoxia related conditions.
- 10 10. The use as claimed in claim 8 wherein the medicament is a vaccine.
11. A method of screening for agents that modulate
 (i) the expression or activity of an SC6 polypeptide as defined in claim 1(i), 2, or 3, or
 (ii) the expression of a nucleic acid molecule as defined in claim 1(ii),
 15 comprising
 comparing the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the presence of a candidate agent with the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, in the absence of the candidate agent or in the presence of a control agent; and
 20 determining whether the candidate agent causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change.
12. The method of claim 11 wherein the expression or activity level of said polypeptide, or the expression level of said nucleic acid molecule is compared with a predetermined reference range.
 25
13. An agent identified by the method of claim 11 or 12, which causes the expression or activity of said polypeptide, or the expression of said nucleic acid molecule, to change.
14. The use of an agent according to claim 13 in the manufacture of a medicament for the
 30 treatment of hypoxia related conditions.
15. An agent according to claim 13 for use in the treatment of hypoxia related conditions.
16. A method of treating hypoxia related conditions, which comprises administering to said
 35 subject a therapeutically effective amount of an agent according to claim 13.
17. The method according to claim 1, 2, 3, 5, 7, 11, 12 or 16 or the use according to claim 8, 9, 10, 14 or 15 wherein the hypoxia related condition is selected from cancer, angiogenesis and angiogenesis dependant conditions.
 40
18. The method or use according to claim 17 wherein the cancer is selected from cervical, colon, renal, lung, uterine, breast or pancreatic cell carcinoma, Burkitt's lymphoma or myeloid or T-cell leukaemia.

Figure 1

1 MATKEKLQCLKDFHKDMVKPSPGKSPGTRPEDEAEGKPPQREKWSSKIDFVLSVAGGFVG 60
61 LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEIIGQYTSEGGITCWEKICPSG 120
121 IGYASVVIVSLLNVYYIVILAWATYYLFQSFQKELPAHCNHSWNTPHCMEDTMRKNVWI 180
181 TISSTNFTSPVIEFWERNVLSLSPGIDHPGSLKWDLALCLLLVWLVCFFCICKGVRGKVV 240
241 YFTATFPFAMLLVLLVRGLTLPGAGRGIKFYLYPDITRLEDPOVWIDAGTQIFFSICLGA 300
301 MTSLGSYNKYKYNYSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQGVADIADVSGPGLA 360
361 FIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPSFLRRREIFIA 420
421 FVCSISYLLGLTMVTEGGMYVFQLFDYYAASGVCLLWVAFFECFVIAWIYGGLYDGIEDM 480
481 IGYRPGPWMKYSWVITPVLCVGCFFISLVKYVPLTYNKTYVSPTWAIGLGWALSSMLCVP 540
541 LVIVIRLCQTEGPFLVRVKYLLTPREPNRWAVEREGATPYNRSRTVMNGALPTHIIVETMM 600

Figure 1

1 MATKEKLQCLKDFHKDMVKPSPGKSPGTRPEDEAEGKPPQREKWSSKIDFVLSVAGGFVG 60
61 LGNVWRFPYLCYKNGGGAFLIPYFIFLFGSGLPVFFLEIIIGQYTSEGGITCWEKICPSG 120
121 IGYASVVIVSLLNVYYIVILAWATYYLFQSFQKELPWAHCNHSWNTPHCMEDTMRKNVWI 180
181 TISSTNFTSPVIEFWERNVLSLSPGIDHPGSLKWDLALCLLLVWLVCFFCICKGVRGKVV 240
241 YFTATFPFAMLLVLLVRGLTLPGAGRGIKFYLYPDITRLEDPOVWIDAGTQIFFSICLGA 300
301 MTSLGSYNKYKYNYSYRDCMLLGCLNSGTSFVSGFAIFSILGFMAQEQQGVADIADVSGPGLA 360
361 FIAYPKAVTMMPLPTFWSILFFIMLLLLGLDSQFVEVEGQITSLVDLYPSFLRRREIFIA 420
421 FVCSISYLLGLTMVTEGGMYVFQLFDYYAASGVCLLWVAFFECFVIAWIYGGLYDGIEDM 420
481 IGYRPGPWMKYSWVITPVLGVGCFIFSLVKYVPLTYNKTYVSPTWAIGLGWALSSMLCVP 540
541 LVIVIRLCQTEGPFLVRVKYLLTPREPNRWAVEREGATPYNSTRVMNGALPTHIIVETMM 600

Figure 2

1	MATKEKLQCLKDFHKDMVKPSPGKSPGTRPEDEAEGKPPQREKWSSKIDFVLSVAGGFVG	60
1	GAATTCCGAAAGCAAGGAGATGGCCACCAAGGAGAAGCTGCAGTGTCTGAAAGATTTCCA	60
61	AAGGACATGGTGAAGCCCTCACCAGGGAAGAGCCCAGGCACGCGGCCTGAGGACGAGGCT	120
121	GGAAAACCTCCGCAGAGGGAGAAGTGGTCTAGCAAGATCGACTTTGTGCTCTCTGTGGCG	180
181	GCTTCGTGGGCTTGGGCAACGTCTGGCGCTTCCCGTACCTCTGCTACAAGAATGGTGGAG	240
241	GTGCGTTTCTCATACCGTATTTTATTTTCTGTGTTGGGAGCGGCCTGCCTGTGTTTCTTG	300
301	GATCATCATAGGCCAGTACACCTCTGAAGGGGGCATCACCTGCTGGGAAAAGATCTGCCC	360
361	GTTCTCTGGTATCGGCTATGCCTCCGTTGTAATTGTGTCCCTCCTGAATGTCTACTACAT	420
421	TCATCCTGGCCTGGGCCACATACTACCTGTTCCAGTCCTTCCAGAAGGAGCTGCCCTGGG	480
481	CTGCAACCACAGCTGGAACACACCTCACTGCATGGAGGACACCATGCGCAAGAACAAGAG	540
541	TGTCTGGATCACCATCAGCTCCACCAACTTCACCTCCCTGTCATCGAGTTCTGGGAGCG	600
601	ACGTGCTGAGCTTGTCCCCTGGAATCGACCACCCAGGCTCTCTGAAATGGGACCTCGCTC	660
661	TGCCTTCTTTTAGTCTGGCTAGTGTGTTTCTTCTGCATCTGCAAGGGCGTCAGGTCCACT	720
721	GAAGGTCGTCTACTTCACAGCCACTTTTTCATTGCGCATGCTCCTGGTGCTGCTGGTCCG	780
781	GGCTGACGCTGCCGGGCGCGGGCCGAGGCATCAAGTTCTATCTGTATCCTGACATCACCC	840
841	CTTGAGGACCCACAGGTGTGGATTGACGCTGGGACTCAGATATTCTTCTCTTATGCCATC	900
901	CCTGGGGGCTATGACCTCGCTGGGGAGCTACAACAAGTACAAGTATAACTCGTACAGGGA	960
961	GTATGCTGCTGGGATGCCTGAACAGTGGTACCAGTTTTGTGTCTGGCTTCGCAATTTTTT	1020
1021	TCCTGGGCTTCATGGCACAAGAGCAAGGGGTGGACATTGCTGATGTGGCTGAGTCAGGTC	1080
1081	GCCTGGCCTTCATTGCCTACCCAAAAGCTGTGACAATGATGCCGCTGCCACATTTTGGT	1140
1141	CCATTCTTTTTTTTATTATGCTTCTCTTGCTTGGACTGGATAGCCAGTTTGTTGAAGTTG	1200
1201	AAGGACAGATCACATCCTTGGTTGATCTTTACCCATCCTTCCTAAGGAAGGGTTATCGTC	1260
1261	GGGAAATCTTCATCGCCTTCGTGTGTAGCATCAGCTACCTGCTGGGGCTGACGATGGTGA	1320
1321	CGGAGGGTGGCATGTATGTGTTTCAGCTCTTTGACTACTATGCAGCTAGCGGTGTATGCC	1380
1380	TTGTGGGTGTCATTCTTTGAATGTTTTGTTATTGCCTGGATATATGGAGGTGATAACCTT	1440
1441	TATGATGGTATTGAGGACATGATTGGCTATCGGCCCGGGCCCTGGATGAAGTACAGCTGG	1500
1501	GTGATCACTCCAGTTCTCTGTGTTGGATGTTTCATCTTCTCGCTCGTCAAGTACGTACCC	1560
1561	CTGACCTACAACAAAACATACGTGTCCCCAACTTGGGCCATTGGGCTGGGCTGGAGCCTG	1620
1621	GCCCTTTCCTCCATGCTCTGCGTTCCCTTGGTCATCGTCATCCGCCTCTGCCAGACTGAG	1680
1681	GGGCCGTTCTTGTGAGAGTCAAGTACCTGCTGACCCCAAGGGAACCCAACCGCTGGGCT	1740
1741	GTGGAGCGCGAGGGAGCCACACCTTACAACCTCTCGCACCGTCATGAACGGCGCTCTCGTG	1800
1800	AAACCGACCCACATCATTGTGGAGACCATGATGTGAGCTCTCTCGGGTCGACGGGGCCGG	1860
1861	CGGCTTTCCTGCTGTTTACTAACATTAGATTACATAGGACCAGGTTTACAGAGCTTTAT	1920
1921	ATTTGCACTAGGATTTTTTTTTTTTTTGTAAATTGTCACAGAAAATGTAATTGTGGGTATGT	1980
1981	GTGCGTGCGT	2040
2041	GGGATATTTTGTACAAAAAGAAAACCCACGGGAAGATGTCCGTGGAGAGGCAGAGCTTTC	2100
2101	ATACTGAATTAGATGTATTTTATGGGAATTTGGTAAATTTTCTTTGTATTTTTTTTTTT	2160
2161	ACATATAAGTATATATACACTTAGAGATTGTCATATACTTTTACCACCTTGAATTGATCTT	2220

2221	CTTGCCAGCAATAGATCTCATTTTCAAAGCAATTCCTTCGGTGCTGTGTAGCTGGCAGAA	2280
2281	AGTTCTGTCCAGTAAACGCAGGATGGAATTTTCCTGGGACTCTACACCCATCTTAAGGTG	2340
2341	GTATACCTTCCAAATCCTGGTTCAGATGGAAGAAATAGCAGGAGAGAGGACCCATTAGCT	2400
2401	GGCAGACCCAGGGGAAGAAAGGAGGGCTGTGAGGAGATACCTCATTAAACTTGGCTTAGT	2460
2461	GAAGAAGAGAGATGCCAAAGGAATGAACCAACCCCTTCACATAAAGGAGACTGGCTGAAGC	2520
2521	TGAATGAGGAGGCCCTATAGCAGAAGTCTGATTCTAAGAGCAGTAGAAACTTGTACCAGA	2580
2581	AGCAAATCCCACCTTTAATTTTGAGATGGTGAGTGGATAGTCAGTAGACCGTCAGAACC	2640
2641	ACTGGCCAGAGAGGGGAGCTGCTAGAGATCCAAGAAGGCTGGCAGGAATGAGGCTCACAAC	2700
2701	TCAGCCTCGCAAGAGGTGGCAGAGGCACAGGAGGCCACAGTCTTCCTGGGGCATTCAG	2760
2761	GCAGAGAAGGAGCAGAGGCTCTCCCGGCAGGAGCTGGGGTCTCAGGGCTCAGATGAGTCT	2820
2821	GTTGCATTTGAATGGGGTCATAGCAGGTTCTGGTCATTCCCCAAGCAACATCTCAGCATC	2880
2881	TCTTAAAGTTGCCTGCAGGAATGAAGCATGACATACCTGTTGAGGGACTAGGGGAGTGGT	2940
2941	GGGGAGGTGAGTGGACCAAAGGATATAGGCCCCAGGCATGCAGATGGGGCCGGTGTCTGGG	3000
3001	GAGGGGTGCTTTCCTTCCTCATCTCCCCACTCCCCACTCTCAGCCTGGGAGACTCCTGCC	3060
3061	AAGCCCTCATTAAGATGCCACCCTGGGCTGCCCTGGCACCTAGCAAGGCACACCAAGAA	3120
3121	CAGCTTTTGAGTCGTATCCTCCACTGGGGAAAGTGCTCCAGTTCAGAACAAGGGCAGCCC	3180
3181	GTGGTGCTGACCTAGGATATAACAAAGCTCTTCACTTCAAACCCCTGCAATAGCTGGGT	3240
3241	TTACAGACATTTACCACCTGGGGACCCAAAAGAGAAGGCCCTAGGAGAGTTTCTAGAAGG	3300
3301	TTGGGATTGTCAGGGTCCTGGCCCCCAGAACTGGCTTGATCAAGGGCCTTATGTGGAGC	3360
3361	AGAGGTTGTCTCTGAACCAGGAGAGAAGGTACTATACCTTTCAAATCCCCAGGGCAGACA	3420
3421	CACCCCCACCCAGCCCCTATTTGGACCTAAACTGTGCCATTTGAACAGTCACTTCCAAGC	3480
3481	TCAGTCTAAATGAAACCGAAACGTGACCACGCACAAAGGCAGTCACTGCTCGAGGGGTGC	3540
3541	AGACCGCAGAATTTTCACAGCAGGGGCTCTTGGAACCTCTGGAAACCCCTTCTTAAATTT	3600
3601	GGGAGGAGGAGTATGCCTTTGGTGTCCTCCCTCCCAAGGGCAATTCTGAACCCCATCTTTG	3660
3661	GCAGGCATACATATTTCACTGTTTCAAAGCTATCTACTCTGCCAAACAACACCCAGTCC	3720
3721	TATTCCAAACTCTCAACGATTCATCTTGTTTCTGTTTCTATGTATTTATGGTTGCCG	3780
3781	TTTGTGTCTGATTTGATTTTACTGTTTTTCCCTGATTTTATGGAGTAGCATTTGTGACCT	3840
3841	GTTTTCTTTGTCTTATATAACTTTAGTAAACTAACCAGTGTCAATGATTGAGGGCAGGT	3900
3901	GGCACGTGGGGAAGAGGGCGGAATTC	3926

Figure 3

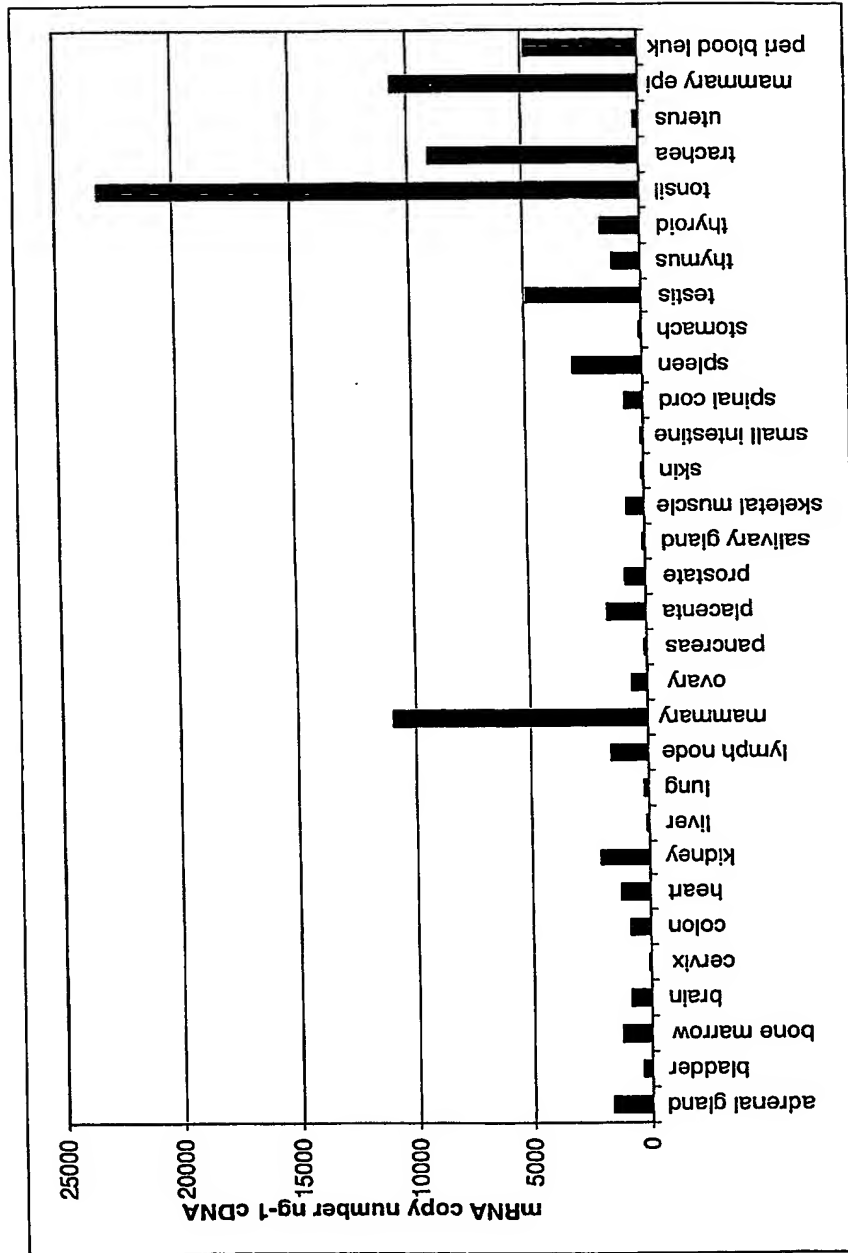


Figure 4

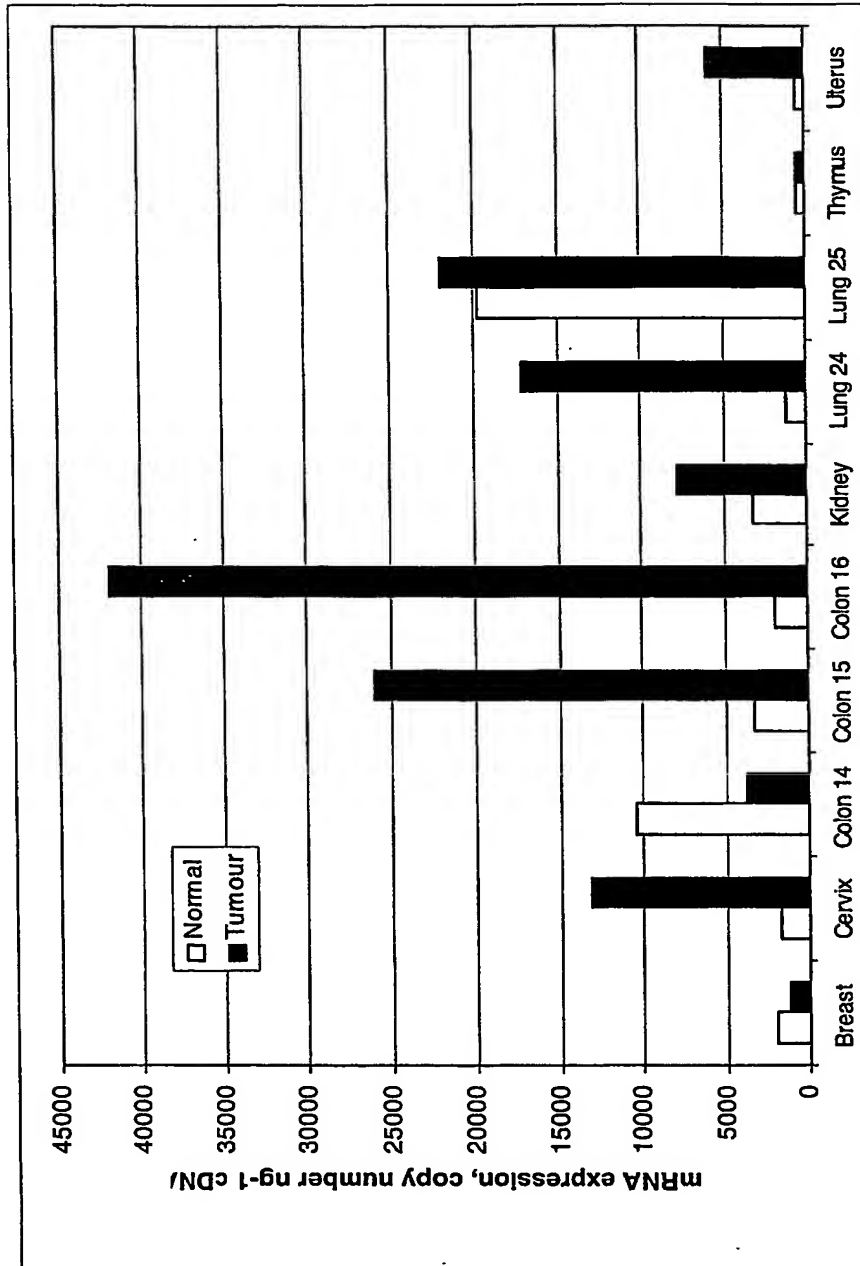


Figure 5

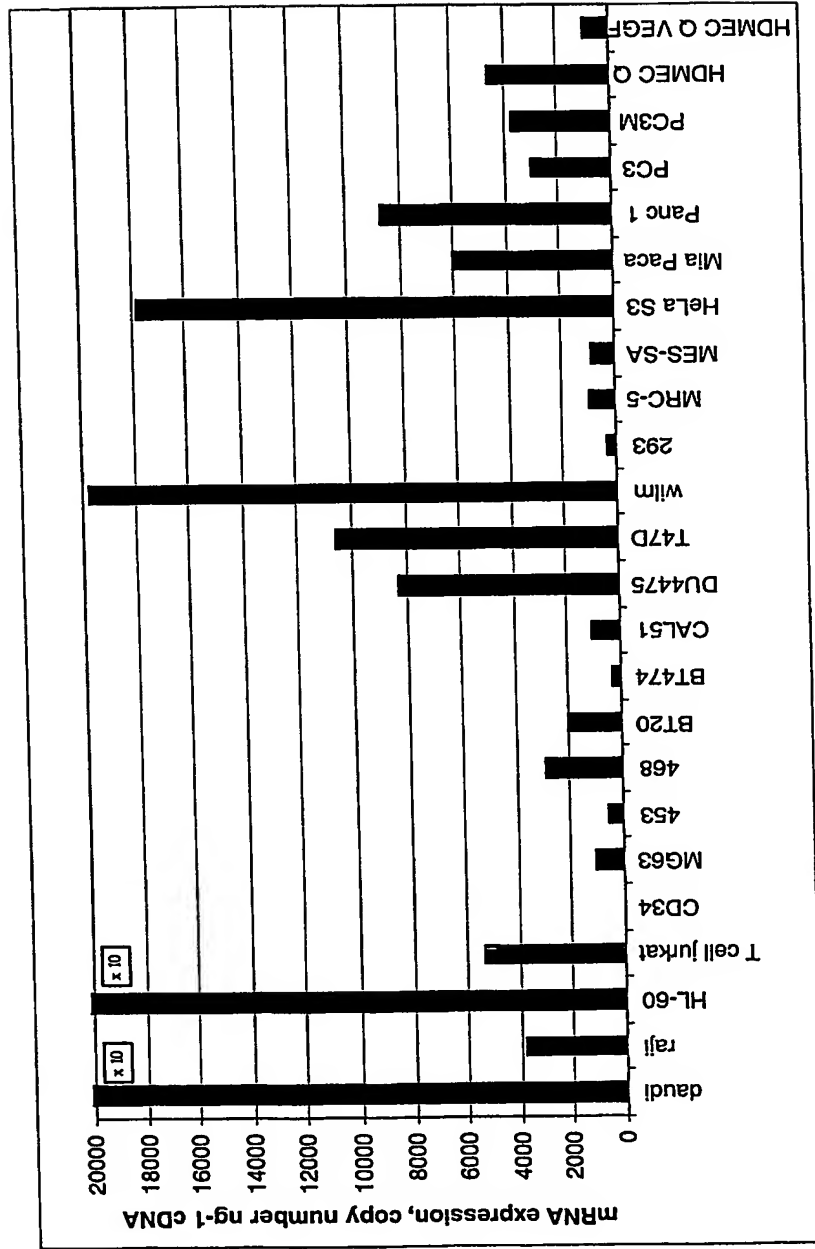


Figure 6

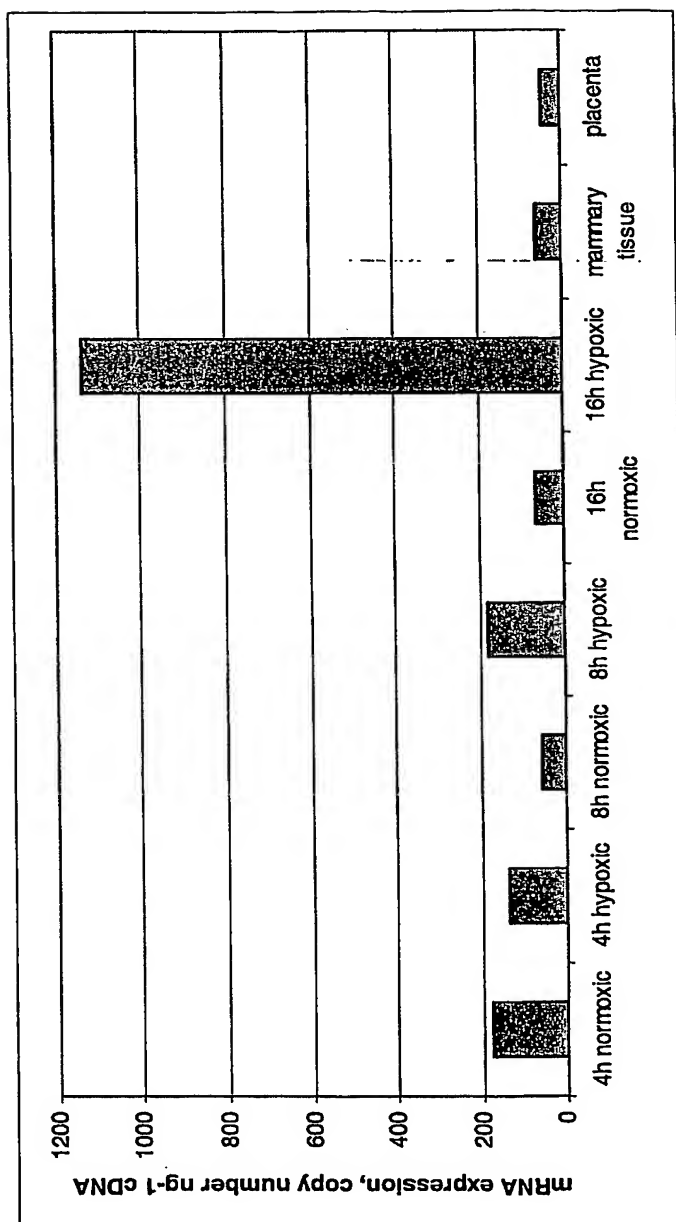
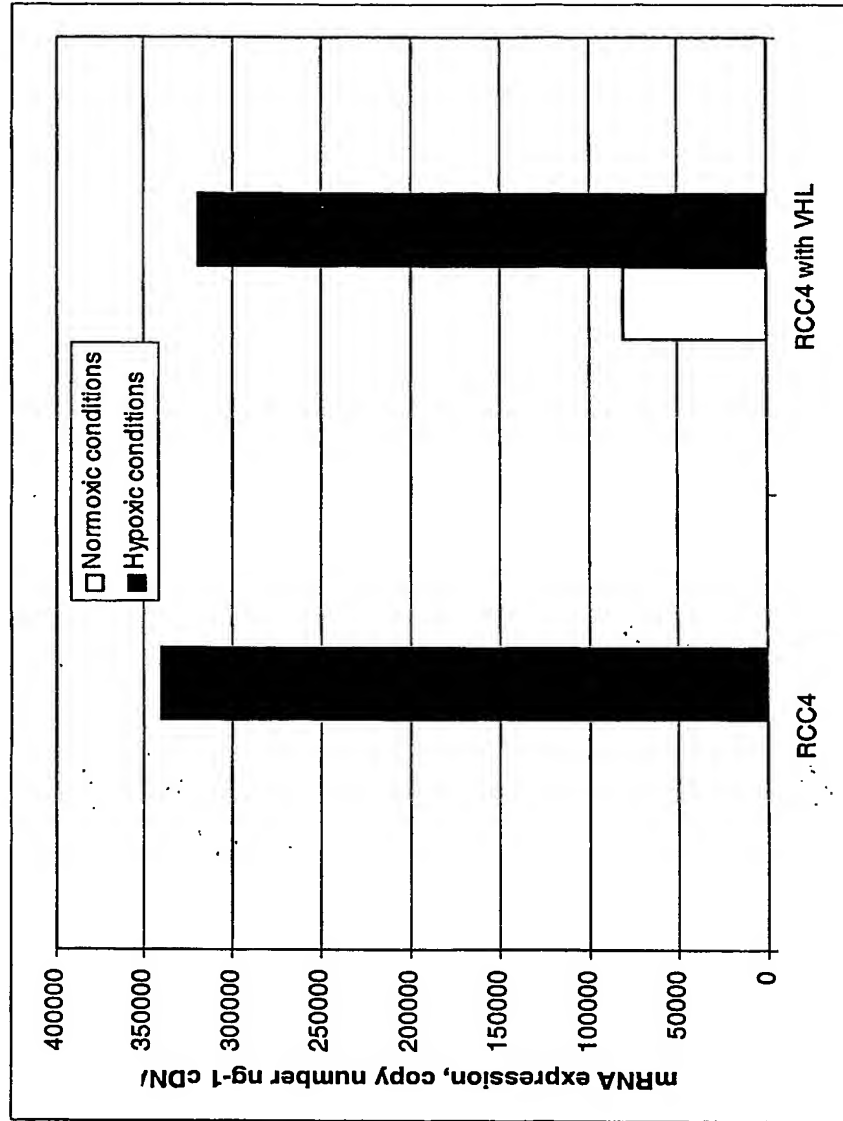


Figure 7



1
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